

CERTIFICATE OF MAILING

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DECLARATION UNDER 37 C.F.R. § 1.132

> Address to: Assistant Commissioner for Patents Washington, D.C. 20231

Attorney Docket	CLON-015
Confirmation No.	
First Named Inventor	Chenchik et al.
Application Number	09/440,829
Filing Date	November 15, 1999
Group Art Unit	1655
Examiner Name	Forman, B.
Title	Long Oligonucleotide Arrays

Sir:

I, Alex Chenchik, am a co-inventor of the above referenced application and an employee of Clontech Laboratories, Inc., the assignee of the above the above referenced application. A copy of my C.V. is enclosed.

I hereby declare the following:

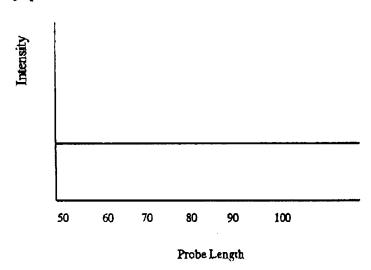
In the working exemplification of the above captioned application, an array is prepared in which the probe lengths range from 50 to 100 nt. See Examples 1 to 5 of the above captioned application.

In Example 6, the hybridization efficiency of each of the different probe lengths on the array was assayed and the results are graphically provided in Fig. 1 of the application.

Prior to conducting the assay of Example 6, it was my expectation, which is the same as with what those of skill in the art would expect, that the hybridization efficiency would be the same regardless of probe length. As such, I expected to obtain results that would graph as follows:

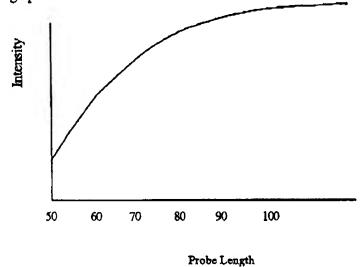
Atty Dkt. No.: CLON-015 USSN: 09/440,829

Expected graph:



Instead of observing the above expected results, the following unexpected results were obtained:

Observed graph:



As such, using a probe length of 50 to 100 nt provides unexpected results.

Atty Dkt. No.: CLON-015

USSN: 09/440,829

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date:

5/25/01

Signature:

Alex Chenchik

enc

C Figure 1

C C.V. of Alex Chenchik

Atty Dkt. No.: CLON-015 USSN: 09/440,829

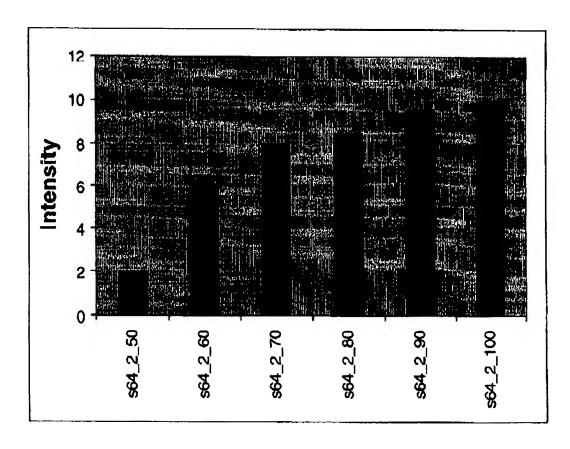


FIGURE 1



CURRICULUM VITAE

ALEXANDR A. TCHENTCHIK, Ph.D.

EDUCATION

Ph.D. degree in Molecular Biology, Institute of Molecular Biology, USSR Academy of Sciences, Moscow, 1982.

Thesis: "Molecular topography of RNA polymerase-lacUV5 promoter complex".

M.S. degree in chemistry. Institute of Fine Chemical Technology, Moscow, 1978. Thesis: "Chemical modification of E.coli RNA polymerase".

EXPERIENCE

1998 - 1999

Director of Array Program, Gene Cloning and Analysis Department, CLONTECH Laboratories, Inc. (1020 East Meadow Circle, Palo Alto, CA94303.

1997 - 1998

Associate Director, Gene Cloning and Analysis Department, CLONTECH Laboratories, Inc. (1020 East Meadow Circle, Palo Alto, CA94303.

Development of new technologies for cDNA/oligonucleotide-based expression arrays, RNA chip, SMART cDNA amplification, RecA-based full-length cDNA cloning.

1994 - 1996

Research Scientist, PCR Group (Cloning II), CLONTECH Laboratories, Inc. (4030 Fabian Way, Palo Alto, CA94303.

Development of new technologies for cDNA library construction, cDNA cloning, genome walking & mapping, cDNA & genomic subtraction, finding differentially expressed genes (RNA fingerprinting, differential display), long-distance PCR, RT-PCR, aptamer selection, RNA isolation.

1992 - 1994

Director of Research Dept.,

"Technogene" company, National Cardiology Research Center, Moscow, Russia; International Distributor of "CLONTECH Laboratories, Inc.", 4030 Fabian Way, Palo Alto, CA 94303, USA. Leader of joint research projects with Clontech:

Leader of joint research projects with Clontech:
Large-scale RNA/DNA purification, New PCR technologies, cDNA library construction, development of new cloning vectors, chemical reagents for oligonucleotide synthesis,

MTW blots. Development of new methods for large-scale RNA isolation, analysis of poly(A)RNA composition, searching of differentially expressed genes, cDNA library construction. Investigation of new nucleotide analogues for reverse transriptase, Tag DNA polymerase, DNA polymerase I.

1984-1992

Senior Scientist,

Genetic Engineering Lab., National Cardiology Research

Center, Moscow, Russia.

Regulation of gene expression at the course of F9 cell

differentiation, in situ hybridization, cell-free system of RNA polymerase II transcription, crystallization of E.coli RNA

polymerase.

1983-1984

Junior Scientist,

Genetic Engineering Lab., National Cardiology Research

Center, Moscow, Russia.

Cloning of plasminogen activator (urokinase) gene,

Regulation of gene expression during F9 cell differentiation

1978-1983

Postgraduate Student,

Institute of Molecular Biology, USSR Academy of Sciences,

Moscow,

Russia.

Investigation of topography of RNA polymerase, lac repressor - lacUV5 promoter complexes by chemical cross-linking approach, Mechanism of RNA polymerase elongation, Development of methods

for large scale purification of RNA polymerase E.coli, DNA

polymerase I E.coli, T4 polynucleotide kinase, T4 DNA ligase, T4

RNA ligase

1974-1978

Undergraduate and M.S. trainee,

Institute of Molecular Biology, USSR Academy of Sciences,

Moscow,

Russia. Chemical modification of E.coli RNA-polymerase.

METHODS

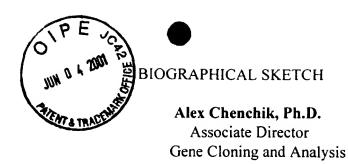
Gene cloning, DNA sequencing, PCR, cDNA library construction/screening, hybridization of nucleic acids, DNA/RNA probes, plasmids/phages microbiology. Chromatography/HPLC/Electrophoresis and others methods of purification/analysis of DNAs/RNAs/Proteins. Cell culture including gene transfection technology.

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- 4. Rozovskaya T.A., Chenchik A.A., Tarusova N.B., Khomutov R.M., Bibilashvilli. Pyrophosphate analogues in pyrophosphorolysis reaction catalyzed by Escherichia coli RNA polymerase. Mol. Biol. (Russia), 1981, v.15, 745-754.
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- 7. Rozovskaya T.A., Chenchik A.A., Bibilashvilli R.Sh. Processive pyrophosphorolysis of RNA by E.coli RNA polymerase. FEBS Letters, 1982, v.137, 100-104.
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 - in human tissues. FEBS Letters, 1993, v.321, 98-101.
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- phosphodiesterase. I. Mol. Biol., 1993, v.27, 67-74.
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- 21. Diatchenko, L., Lau, Y-F, C., Campbell, A., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E., and Siebert, P.D. (1996) Suppression Subtractive Hybridization: A method for generation of differentially regulated or tissue-specific cDNA probes and libraries. Proc. Natl. Acad. Sci.. USA 93:6025-6030.
- 22. Gurskaya, N., Diatchenko, L., Chenchik, A., Siebert, P., Khaspekov, G., Lukyanov, K., Vagner, L., Ermolaeva, O., Lukyanov, S., and Sverdlov, E. (1996) Equalizing cDNA subtraction based on selective suppression of polymerase chain reaction: cloning of jurkat cell transcripts induced by phytohemaglutinin and phorbol 12-myristate13-acetate. Analytical Biochemistry 240:90-97.
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- Lukyanov, K., Diatchenko, L., Chenchik, A., Nanisetti, A., Siebert, P., Usman, N., Matz, M., and Lukyanov, S. (1997) Construction of cDNA libraries from small amounts of total RNA using the supression PCR effect. Biochem. Biophys. Res. Comm., 230: 285-288.
- 26. Zhang, W., Chenchik, A., Chen, S., Siebert, P. and Rhee, C.H. (1997) Molecular Profiling of human gliomas by cDNA expression array. J. of Genet. Medicine., 1:57-59.
- 27. Chenchik, A., Zhu, Y.Y., Diatchenko, L., Hill, J. and Siebert, P.D. (1998) Generation and Use of High-Quality cDNA from Small Amounts of Total RNA by SMART PCR. In RT-PCR Methods for Gene Cloning and Analisis. Ed. by P. Siebert and J. Larrick, BioTechniques Books, Natick, MA., p.305-320.

- 28. Diatchenko, L., Chenchik, A. and Siebert, P. Suppression Subtractive Hybridization: A method for Generating Subtracted cDNA Libraries Starting from Poly(A+) or Total RNA. In RT-PCR Methods for Gene Cloning and Analisis. (1999) Ed. by P. Siebert and J. Larrick, BioTechniques Books, Natick, MA., p.213-238.
- 29. Chen, S.S., Chenchik, A., Lukianov, K.A. and Siebert, P. Improved Technique for walking in Uncloned Genomic DNA. In RT-PCR Methods for Gene Cloning and Analisis. (1999) Ed. by P. Siebert and J. Larrick, BioTechniques Books, Natick, MA., p.289-302.



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SCIENTIFIC BACKGROUND

Dr. Alex Chenchik obtained his Ph.D. degree in Molecular Biology (1982) in Professor Robert Bibilashvili's laboratory at the Institute of Molecular Biology (Moscow, Russia) where he worked for three years as a post graduate student. His thesis, "Molecular topography of RNA polymerase-lacUV5 complex", examined the three-dimensional structure of complexes of the *Escherichia coli* RNA polymerase with lac promoter.

After receiving his Ph.D., Dr. Chenchik together with Professor Bibilashvili setted up a new laboratory of Genetic Engineering in National Cardiology Research Center (Moscow, Russia) in 1984. As a research scientist and later as a senior research scientist he has been involved in cloning and generation of *E. coli* overproducer strain of human urokinase for pharmaceutical industry, regulation of gene expression in the course of embrional carcinoma cell differentiation, searching and investigation of new nucleotide analogues for reverse transcriptase, Taq DNA polymerase and DNA polymerase I. He developed a several novel methods for large-scale RNA isolation, searching of differentially expressed genes based on reverse transcriptase extension assay, cDNA library construction, cDNA subtraction and "hot-start" PCR based on using monoclonal antibodies against Taq DNA polymerase.

In April 1994, Dr. Chenchik joined PCR group at CLONTECH as the Research Scientist in charge of the development of PCR-based products for gene cloning and analysis. In January 1995 he introduced the MarathonTM cDNA Amplification kit and latter Marathon-Ready cDNAs for fast PCR-based cloning of full-length cDNAs. Dr. Chenchik has been participate in the development of the TaqStartTM antibodies technology for "hot-start" PCR, AdvantageTM PCR Amplification Kits for long-distance PCR, PCR-SelectTM cDNA Subtraction Kit for selective amplification of differentially expressed genes, Promoter FinderTM for PCR-based cloning of promoter regions, CapFinderTM PCR cDNA Library Construction Kit for generating high-quality cDNA libraries from nanogram amount of total RNA and AtlasTM cDNA Expression Array for high throughput gene expression analysis. Dr. Chenchik is author of 27 scientific publications and 5 patents. Currently he is the leader of the group of 30 people responsible for development of novel technologies for cDNA cloning and expression analysis at CLONTECH.

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- 1. Chenchik A.A., Bibilashvilli R.Sh. Chemical modification of RNA polymerase by dimethylsulfate. Mol. Biol. (Russia),1977, v.11, 403-409.
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 - in human tissues. FEBS Letters, 1993, v.321, 98-101.
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